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Gas Chromatography-Mass Spectrometry Characterization and Docking Analysis of Zinc Oxide Nanoparticles from *Canavalia rosea*

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Abstract: Nanotechnology is a flourishing domain of recent research associated with the creation of nanoparticles. We explored a sand dune, *Canavalia rosea* for the synthesis of zinc oxide nanoparticles through a green synthesis process from methanol extract of the leaf and stem extract of *C. rosea*. The ZnO oxide nanoparticles were characterized by Gas Chromatography-Mass Spectrometry (GC-MS). The compounds obtained from GC-MS with strong peaks were screened for the docking procedure. The docking analysis of hit compounds from methanol extract of both leaf and stem extract was analyzed with caspase-9, TNF-alpha, HER-2, and ER-alpha receptor proteins to validate the best binding interactions.

Keywords: Docking analysis, Zinc oxide-nanoparticles, *Canavalia rosea*, Green synthesis, Gas Chromatography-Mass Spectrometry, Sand dune

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Introduction

Nanotechnology is an advancing field of scientific research that deals with the fabrication of nanoparticles with an average particle size ranging from 0.1 to 100 nm (Singh *et al.*, 2021). Plants are commonly used as resources for nanoparticle (NP) production, as they can potentially synthesize bulk amounts of highly stable, nanoparticles in different shapes on a large scale. (Jamdagni *et al.*, 2018; Vinotha *et al.*, 2019; Ali *et al.*, 2021;). The synthesis of nanoparticles by

biological method or green synthesis involves the bioactive constituents or phytochemicals which act as capping or reducing agents, thereby facilitating the formation of nanoparticles (Elumalai and Velmurugan, 2015; Murali *et al.*, 2017; Rajakumar *et al.*, 2018; Anandan *et al.*, 2019; Hemanth kumar *et al.*, 2020; Pillai *et al.*, 2020). Few researchers have realized that plants and microbial organisms are reliable sources for nanoparticle synthesis (Shafey, 2020; Klink *et al.*,

2022; Cuong *et al.*, 2022; Ettadili *et al.*, 2022; Lomeli-Rosales *et al.*, 2022; Majeed *et al.*, 2022; Mustapha *et al.*, 2022; Najafi *et al.*, 2022). Among the nanoparticles providing greater efficiency, the nanoparticles fabricated from zinc and silver are widely studied (Cameron *et al.*, 2022).

Zinc ions play a pivotal role in intracellular bacteriological toxicity, disorganizing their cell membranes (Mandal *et al.*, 2022). ZnO-NPs are applied in the following industries namely therapeutics, biological and electronic sensors, medical implants, gene transfer, and nanoformulation in the discovery, communication, and wastewater treatment (Wiesmann *et al.*, 2020; Shaba *et al.*, 2021; Qin *et al.*, 2021). The cytotoxic effect of ZnO-NPs on human cancer cells, via apoptosis leading to cell death was validated (Weismann *et al.*, 2019). Moreover, substantial ROS generation directs the process of enhanced oxidation, which in turn enriches the antimicrobial property of the zinc oxide nanoparticles (Sirelkhatim *et al.*, 2015).

Zinc oxide is an exceptional metal with extensive semiconductor and catalytic properties (Wang, 2004; Nagajyothi *et al.*, 2015; Stan *et al.*, 2016; Gao *et al.*, 2020; Ali *et al.*, 2021). Many investigations about ZnO revealed the antimicrobial efficacy of this metal and stated its capability of scavenging free radicals (Nagajyothi *et al.*, 2015). The biocompatible nature of ZnO enabled its application, across several industries like electronic systems, skincare, and pharmaceutical targets and also the biomedical industry (Agarwal and Shanmugam, 2019; Ali *et al.*, 2021; Chunchegowda *et al.*, 2021). The use of green methods for the synthesis of ZnONPs has attracted much attention due to their simplicity, low cost, and environment friendly (Mittal *et al.*, 2013; Elumalai and Velmurugan, 2015). Due to their specific physical and chemical properties, the ZnONPs are widely used in several fields (Ruszkiewicz *et al.*, 2017). In the rubber industry, ZnONPs are used to enhance the toughness and intensity of the rubber (Sahoo *et al.*, 2000). In the textile industry, zinc nanoparticles are utilized,

which improve the antibacterial, and deodorant activities of woven fabrics (Fouda *et al.*, 2018).

Several earlier studies have highlighted the notorious phytochemicals proven to be present in the extracts of *C. rosea* (Huang *et al.*, 2012; Niu *et al.*, 2014). The phytochemical analysis performed with methanol extract showed the occurrence of saponins, flavonoids, alkaloids, tannins, phlobatannins, cardiac glycosides, and phenolic compounds. Also, the methanolic leaf extract of *C. rosea* exhibits antibacterial activity (Prabhu *et al.*, 2010). Likewise, the plant was found to possess remarkable medicinal properties. This plant has been selected for the study to explore the constituents present in methanol extract through GC-MS analysis. The compounds identified from GC-MS were subjected to virtual screening analysis with selected receptor proteins. The virtual screening of hitlist compounds isolated from methanolic extract of Leaf-ZnONP and Stem-ZnONP of *C. rosea* with the receptor proteins 1A52 (Estrogen receptor- α), 3PP0 [Human Epidermal Growth Factor (Her-2)], 1TNF- α (Tumor Necrosis Factor- α) and 1NW9 (Caspase 9) to evaluate the binding interactions.

Materials and Methods

Plant collection and validation:

A psammophytic plant, *Canavalia rosea*, was chosen for this research, and bunched from the coastal area of the Cuddalore district. The herbarium specimen of the plant was authenticated and confirmed as *Canavalia rosea* by the Rapinet Herbarium of the Department of Botany, St. Joseph Autonomous College in Tiruchirappalli, India. The specimen number is 2916.

Green synthesis of Zinc nanoparticles from leaf and stem extract:

Different volumes of *Canavalia rosea* methanol extracts of leaf and stem were mixed with 6 g of zinc nitrate hexahydrate. The mixture was allowed to dissolve in a magnetic stirrer. Once the solution was completely dissolved, the solution was boiled at 60-65°C for 1 h in a magnetic stirrer until a deep

yellow-color paste was formed. The paste was transferred to a ceramic crucible cup and heated in a furnace at 450°C for 1 h. The zinc oxide nanoparticles synthesized from leaf extract (Leaf-ZnONP) displayed green color, whereas the ZnO nanoparticles synthesized from stem extract (Stem-ZnONP) presented off-white or yellow color. The dried powder form of ZnONPs was stored in sterile Eppendorf tubes.

Characterization of nanoparticles:

GC-MS analysis:

GC-MS is a widely used analytical technique for qualitative and quantitative estimation through which volatile compounds are analyzed. In this method, there is a gas and a liquid phase. The liquid phase is stationary whereas the gas phase is a mobile phase. Compounds to be verified are also in the mobile phase, with a carrier gas usually helium, hydrogen, or argon. The chemical constituents are separated depending on the migration rate into the liquid phase. A higher percentage of the ingredients will lead to faster migration in the liquid phase.

Quantitative and qualitative analysis of *Canavalia rosea* (leaf and stem) extracts were analyzed using GC-MS (Model Clarus 680, Perkin Elmer). The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min.

The samples were injected at a split of 10:1; The injector temperature was set at 260°C during the chromatographic run. 1 µl of extract sample was injected into the instrument. The oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min⁻¹; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature of 230 °C; ion source temperature of 230 °C; and ionization mode electron impact at 70 eV, a scan time of 0.2 sec, and scan interval of 0.1

sec. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS NIST (2008) library. TurboMass Ver 5.4.2 software was used for data analysis.

Docking analysis:

Protein and Ligand preparation:

The selection of these specific protein targets for the docking prediction was followed as illustrated by Darwati *et al.* (2021). Autodock Vina was utilized for the molecular docking analysis of the proteins and the selected compounds. The removal of water molecules was ensured before the autodock procedure. The ligands are the compounds selected from the ZnONPs synthesized from leaf and stem extracts of *Canavalia rosea*. All the compounds selected for docking analysis were screened for Lipinski's rule of 5 (Ro5), which includes the following rules to be considered for the selection criteria of oral-drug candidates: (1) Molecular mass should be lesser than ≤500, (2) Calculated octanol/water partition coefficient (CLogP) ≤ 5, (3) Number of hydrogen bond donors ≤ 5, and (4) Number of hydrogen bond acceptors ≤ 10. The hitlist compounds identified by GC-MS from (the MeOH extract of *C. rosea*) of Leaf- ZnONP and Stem-ZnONP, were 5 and 4, respectively. Only Methane dichloro nitro and Acetic acid dichloro were the compounds, recognized from Leaf-ZnONP and Stem-ZnONP that fulfill Lipinski's rule of 5.

Molecular docking procedure:

A molecular docking protocol was followed to validate the binding interaction of the hit list compounds obtained in GC-MS, with the protein targets. The three-dimensional structures of the protein target sequence, caspase 9, estrogen receptor, tumor necrosis factor, and human epidermal growth factor are fetched from Protein Data Bank (PDB) database corresponding to ID 1NW9, 1A52, 1TNF-α, and 3PP0, respectively, and used for docking procedure. The 3D structures were obtained from the RCSB Protein Data Bank (<https://www.rcsb.org/>) in PDB format. Auto

dock tools were used to analyze the binding affinity of the respective compounds exhibited by silver nanoparticles processed from leaf and stem extract.

Results

GC-MS compounds of Leaf extract + ZnONPs:

GC-MS analysis identified the presence of the following compounds in the nanoparticles fabricated using zinc oxide and leaf extract (Leaf extract + ZnONPs). The zinc oxide nanoparticles fabricated from leaf extract displayed a total of 33 compounds, from which 5 major hit compounds were shortlisted from GC-MS analysis (Fig. 1). The major compounds reported in the sample are listed as follows: Trichloromethane, Methane-dichloronitro, Methane, Oxybis (dichloro), Methane Bromodichloro, and Benzoic acid, 2-(1-oxypropyl). Table 1 reveals the hit list compounds identified from the zinc oxide nanoparticles synthesized from the leaf extract of *Canavalia rosea*.

GC-MS compounds of Stem extract + ZnONPs:

ZnO nanoparticles generated from stem extract exposed aggregates of 12 metabolic compounds, from which the five compounds with prominent peaks were selectively chosen for the study (Fig. 2). The major peaks from GC-MS results of Stem + ZnONPs represent the following compounds: Dichloroacetaldehyde, Methylene chloride, Acetic acid dichloro, and Chloromethane sulfonyl chloride. Table 2 reveals the hit list compounds identified from the zinc oxide nanoparticles synthesized from the leaf extract of *Canavalia rosea*.

Docking results of Leaf extract - ZnO Nanoparticle:

The docking analysis was performed with the five hit compounds, as follows: Trichloromethane, Methane-dichloronitro, Methane, Oxybis (dichloro), Methane Bromodichloro, and Benzoic acid, 2-(1-oxypropyl). All these compounds get fragmented during the docking process, except methyl dichloro nitro. So, we chose this major metabolic compound for the docking process. The

methane, dichloro nitro was subjected to a docking study, with proteins namely 1TNF- α , 1NW9, 3PP0, and 1A52 to predict the binding interactions, illustrated in Table 3.

Docking with estrogen receptor:

The binding interactions with methane dichloro nitro were assessed with estrogen receptor, 1A52. The best binding affinity was observed with -3.9 kcal/mol, between the protein and the ligand, defining the generation of a covalent hydrogen bond with Lys449 residue. Figure 3 illustrates the best binding pose of 1A52 with this compound.

Docking with Caspase-9:

Methane dichloro nitro was evaluated for its interaction efficiency with caspase-9 protein, 1NW9. The best binding affinity was observed with -3.2 kcal/mol, between the protein and the ligand. The formation of a single covalent hydrogen bond was observed with the binding site of Trp323 residue, which is demonstrated in the Figure 4.

Docking with Tumour Necrosis factor- α :

The binding interactions between methane dichloro nitro and Tumor necrosis factor, 1 TNF- α were assessed using the protein sequence retrieved from the Uniprot database. The best binding affinity was observed with -3.3 kcal/mol, between the protein and the ligand (1A52) as displayed in Figure 5. The bonding type was evident through the two covalent hydrogen bonds with Ser147(Serine) residue, and Asn34 (Asparagine) residue, along with one carbon-hydrogen bond with Arg32 (Arginine) residue.

Docking with Human epidermal growth factor HER-2:

Human epidermal growth factor HER-2, protein (3PP0) displayed an efficient binding affinity (-3.6 kcal/mol), with methane dichloro nitro. Three covalent hydrogen bonds are noticed at binding sites Leu785 residue, Thr862 residue, and Ser783 residue, as illustrated in Figure 6.

The docking experiments with the compound (Methane dichloro nitro) and various receptor

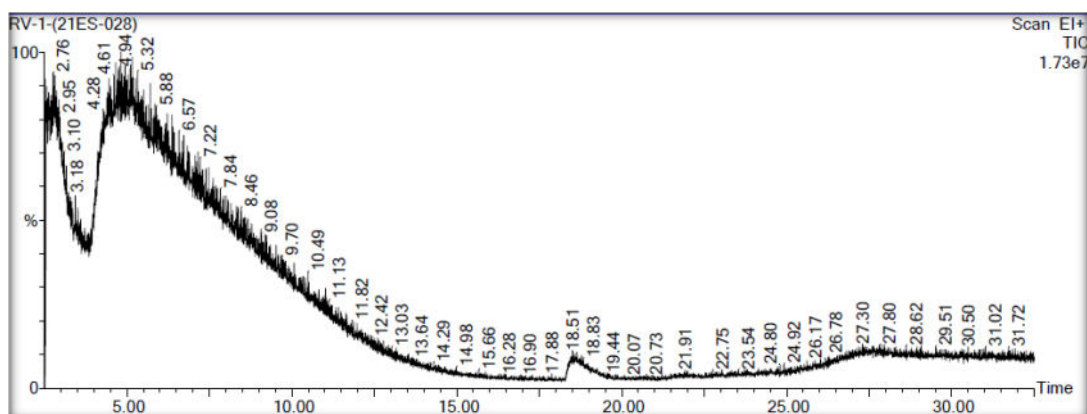


Fig. 1: Chromatogram of zinc nanoparticles synthesized from leaf extract (Leaf + ZnONP). The major peaks are recognized, and the hit list compounds are depicted in Table 1.

Table 1: Compounds identified from (MeOH extract) Leaf extract + ZnONP sample in GC-MS analysis

S. No.	RT	Name	Structure	Mol. wt. (g/mol)	Mol. formula
1.	4.459	Trichloro- methane		118	CHCl ₃
2.	5.029	Methane- dichloro-nitro		129	CHCl ₂ NO ₂
3.	6.575	Methane, Oxybis (dichloro)		182	C ₂ H ₂ Cl ₄ O
4.	6.895	Methane Bromodichloro		162	CHBrCl ₂
5.	18.54	Benzoic acid, 2- (1-oxypropyl)		178	C ₁₀ H ₁₀ O ₃

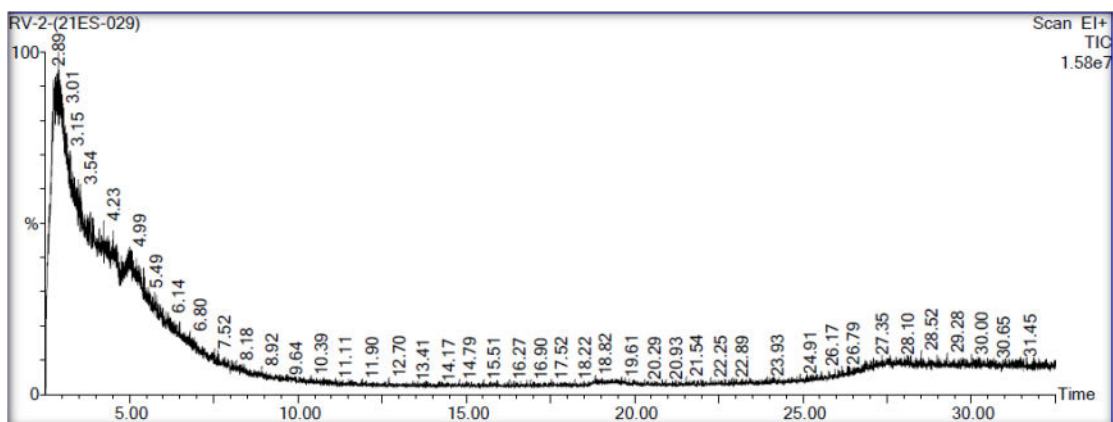


Fig. 2: Chromatogram of zinc nanoparticles synthesized from stem extract (Stem + ZnONP). The major peaks are recognized, and the hit list compounds are depicted in Table 2.

Table 2: Compounds identified from (MeOH extract) Stem extract + ZnONP sample in GC-MS analysis

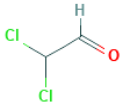
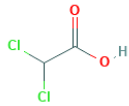
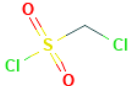
S. No.	RT	Name	Structure	Mol. Wt (g/mol)	Mol. formula
1.	3.479	Dichloroacetaldehyde		112	C ₂ H ₂ OCl ₂
2.	3.844	Methylene chloride		84	CH ₂ Cl ₂
3.	4.229	Acetic acid dichloro		128	C ₂ H ₂ O ₂ Cl ₂
4.	4.994	Chloromethane sulfonyl chloride.		148	CH ₂ O ₂ Cl ₂ S

Table 3: Docking analysis of leaf extract + ZnONPs and interpretation of binding interactions between Methane dichloro nitro (hit compound from Leaf extract +ZnONP) with various protein targets 1A52, 1TNF- α , 1NW9, and 3PP0.

S. No.	Compound	Docked Protein	Binding energy	Covalent Hydrogen bonds		Carbon-Hydrogen bond	
1.	Methane dichloro nitro	1A52	-3.9	1	Lys449	-	-
2.		1TNF - α	-3.3	2	Ser147, Asn34	1	Arg32
3.		1NW9	-3.2	1	Trp323	-	-
4.		3PP0	-3.6	3	Leu78, Thr862, Ser783	-	-

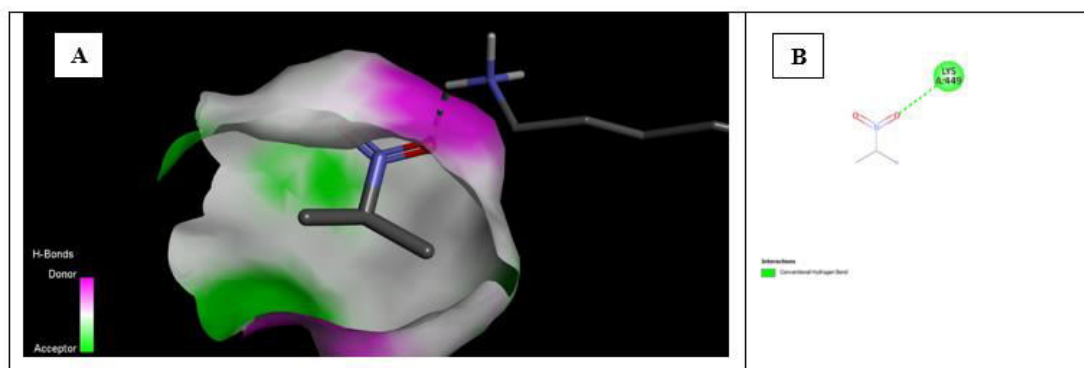


Fig. 3: Illustrative representation of docking results of a highly ranked pose of major interactions with the pocket binding site of Estrogen receptor- α (PDB ID:1A52) with hitlist compound Methane dichloronitro isolated from Leaf+ZnONP. (A) represents the 3D image, and (B) represents the 2D image of the conventional hydrogen bond, respectively.

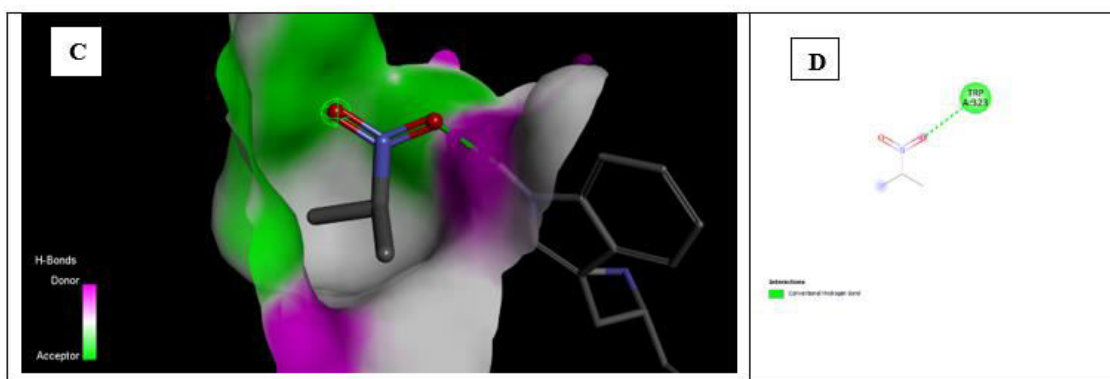


Fig. 4: Pictorial representation of docking results of a best-ranked pose of major interactions with the pocket binding site of Caspase-9 protein (PDB ID:1NW9) with hitlist compound Methane dichloronitro isolated from Leaf+ZnONP. (C) represents the 3D image, and (D) represents the 2D image of the conventional hydrogen bond, respectively.

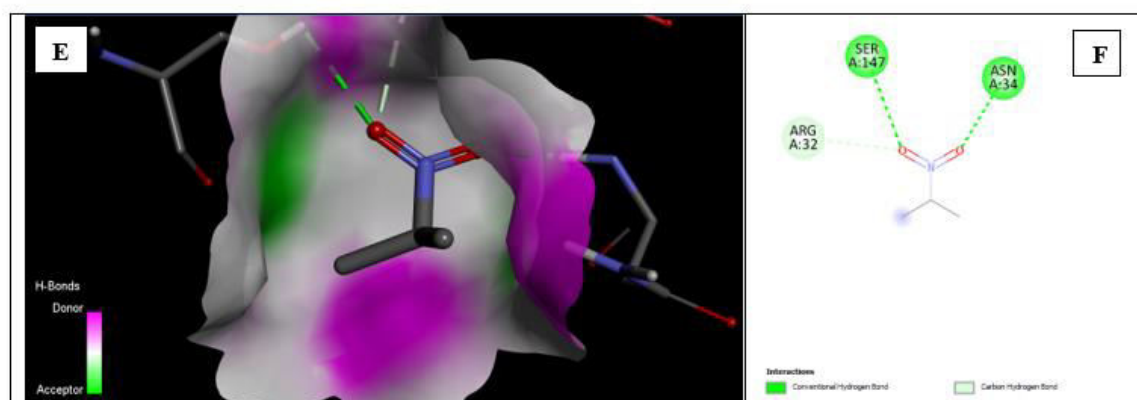


Fig. 5: Graphic representation of docking results of a best-ranked pose of major interactions with the pocket binding site of Tumour necrosis factor (PDB ID:1TNF α) with hitlist compound Methane dichloronitro isolated from Leaf+ZnONP. (E) represents the 3D image, and (F) represents the 2D image of the conventional hydrogen bond, respectively.

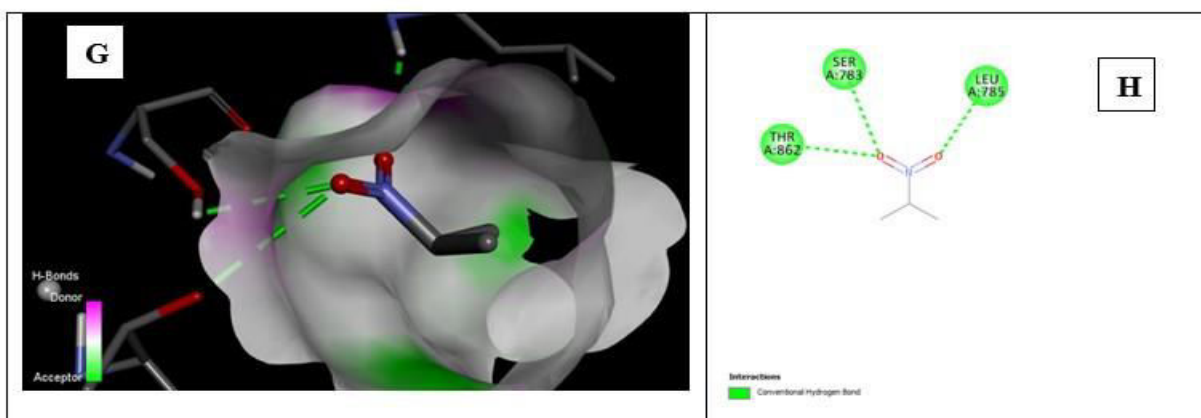


Fig. 6: Illustrative representation of docking results of a best-ranked pose of major interactions with the pocket binding site of Human epidermal Growth Factor-2 (HER-2) (PDB ID:3PP0) with hitlist compound Methane dichloronitro isolated from Leaf+ZnONP. (G) represents the 3D image, and (H) represents the 2D image of the conventional hydrogen bond, respectively.

proteins (1A52, 1TNF α , 1NW9, and 3PP0) confirmed that the compound methane-dichloro nitro demonstrates higher binding efficiency against the receptor protein 1A52, which was evident from the binding affinity of -3.9Kcal/mol.

Docking results of Stem extract -ZnO nanoparticles:

The zinc oxide nanoparticles produced from stem extract exhibit 12 compounds from which 4 hit compounds were confined from GC-MS analysis. The docking procedure was executed with the four compounds, Dichloroacetaldehyde, Methylene chloride, Acetic acid dichloro, and Chloromethane sulfonyl chloride. Among these four compounds, other than Acetic acid dichloro, all the compounds showed fragmentation during the docking procedure, similar to the fragmentation noticed in leaf extract -zinc nanoparticles. The same protein targets analyzed for leaf extract are followed for the stem extract-zinc nanoparticles, illustrated in Table 4.

Docking with estrogen receptor:

The binding interactions with acetic acid dichloro were assessed with estrogen receptor, 1A52, as represented in Figure 7. The best binding affinity was observed with -3.9 kcal/mol, between the protein and the ligand, defining the generation of covalent hydrogen bonds with Lys520 residue, and two carbon-hydrogen bonds with Gly420 residues.

Docking with Caspase-9:

The binding interactions between acetic acid dichloro and tumor necrosis factor, 1TNF- α were examined using the protein sequence retrieved from the Protein Data Bank database. The best binding affinity was observed with -3.5 kcal/mol, between the protein and the ligand (1TNF- α) (Fig. 8). The bonding type was evident through the three covalent hydrogen bonds with Thr308, Asp309, Gly306 residues and one carbon-hydrogen interaction at Lys 311 residue.

Docking with Tumour Necrosis factor- α :

Acetic acid dichloro was evaluated for its interaction efficiency with caspase-9 protein,

1NW9. The best binding affinity was observed with -4.2 kcal/mol, between the protein and the ligand. The formation of four covalent hydrogen bonds was observed with the binding site of Lys65, Leu142, Gly24, and Asp140, as displayed in Figure 9.

Docking with Human epidermal growth factor HER-2:

Human epidermal growth factor HER-2, protein (3PP0) exposed the efficient binding affinity (-3.7 kcal/mol), with acetic acid dichloro, as shown in Figure 10. Two covalent hydrogen bonds are noticed at binding sites Lys 921 residue, and Tyr923 residue, along with a single carbon-hydrogen bond, corresponding to a binding residue, Pro942.

Discussion

Zinc oxide metals are commonly used in the fabrication of nanoparticles, through the biological method, as they are efficient and biocompatible. The green synthesized ZnONPs from the floral extract of *Clitoria ternatea* could be used as an alternative medication for the diagnosis of biofilm-related oral infection (Lahiri *et al.*, 2022). A study showed the inhibitory activity of ZnONPs against all tested bacterial strains, and *Candida albicans* (Klink *et al.*, 2022). The Phyto-fabricated ZnONPs from *Ipomoea obscura* displayed genotoxicity and cytotoxicity against *Allium cepa* meristem and HT-29 cells, supporting the ZnONPs could be a potent molecule for cytotoxicity (Murali *et al.*, 2021). A study reported the efficient biological activity against colon cancer by exhibiting zinc oxide nanoparticles of *croton tiglium* seeds (Aboulthana *et al.*, 2022). It has been determined that the extract of *C. sativum* possesses reducing properties of zinc metal salt and the formation of nanostructures by forming a ZnO -phenolate complex (chelating effect), resulting in the improved germination process (Asmat-Campos *et al.*, 2022).

Molecular docking and simulations are the appropriate tools to authenticate the binding interactions between the ligand and the receptor,

Table 4: Docking analysis of Stem extract + ZnONPs and interpretation of binding interactions between Acetic acid dichloro (Hit compound from Stem extract + ZnONP) with various protein targets 1A52, 1TNF- α , 1NW9, and 3PP0

S. No.	Compound	Docked Protein	Binding energy	Covalent Hydrogen bonds		Carbon- Hydrogen bond	
1.	Acetic Acid Dichloro	1A52	-3.9	1	Lys520	2	Gly420
2.		1TNF - α	-3.5	4	Lys65, Leu142, Gly24, Asp140	-	-
3.		1NW9	-4.2	3	Thr308, Asp309 Gly306	1	Lys311
4.		3PP0	-3.7	2	Lys921, Tyr923	1	Pro942

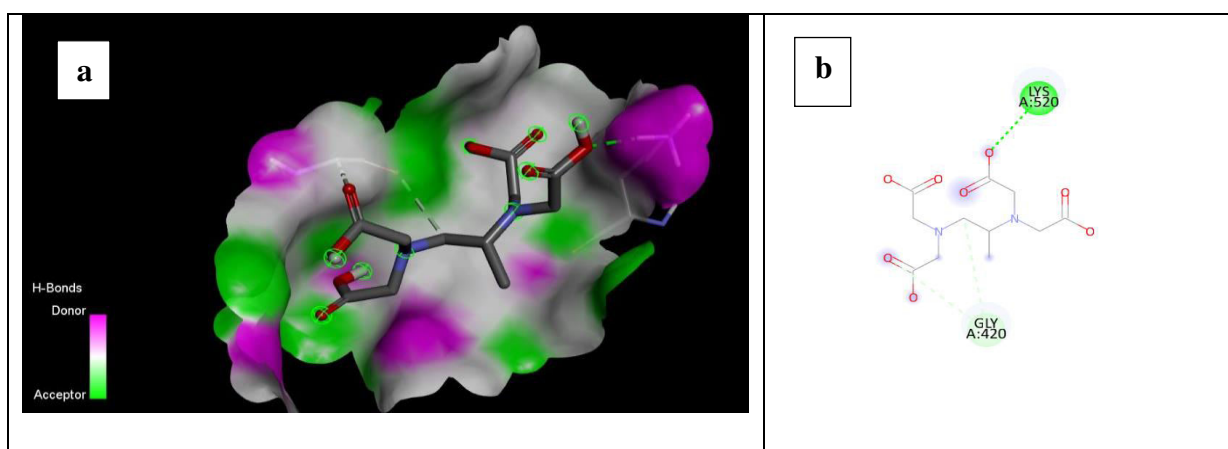


Fig. 7: Illustrative representation of docking results of a highly ranked pose of major interactions with the pocket binding site of Estrogen receptor- α (PDB ID:1A52) with hitlist compound Acetic acid dichloro isolated from Stem + ZnONP. (a) represents the 3D image, and (b) represents the 2D image of the conventional hydrogen bond, respectively.

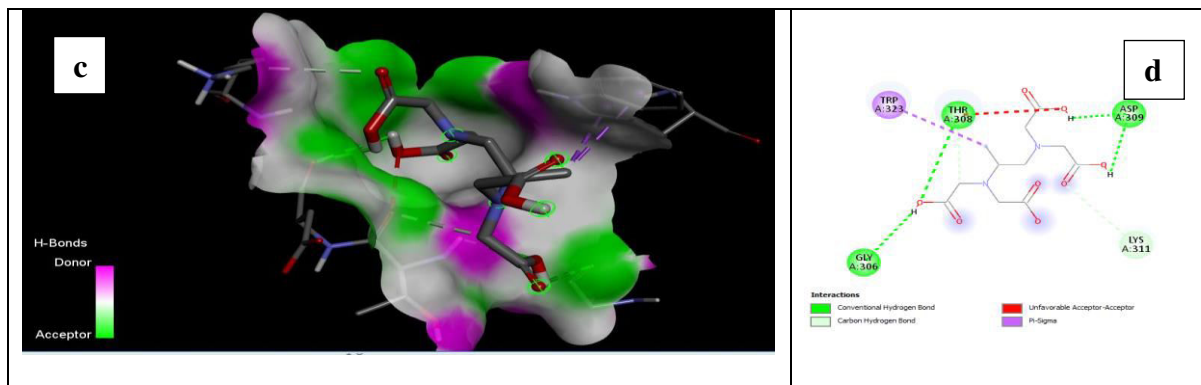


Fig. 8: Illustrative representation of docking results of a best-ranked pose of major interactions with the pocket binding site of Caspase-9 protein (PDB ID:1NW9) with hitlist compound Acetic acid dichloro isolated from Stem + ZnONP. (c) represents the 3D image, and (d) represents the 2D image of the hydrogen bonds, respectively.

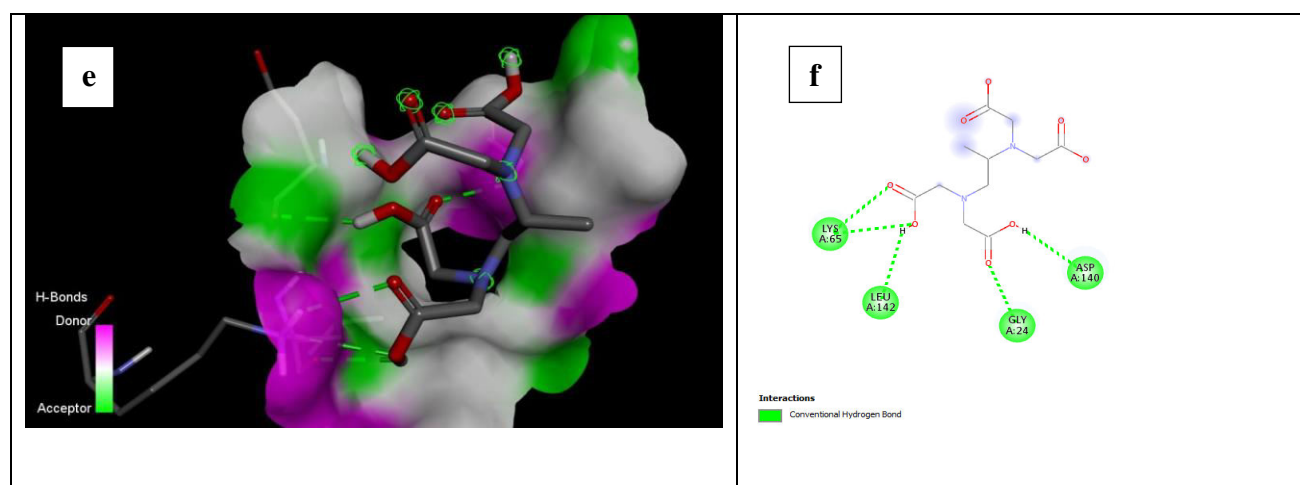


Fig. 9: Illustrative representation of docking results of a best-ranked pose of major interactions with the pocket binding site of Tumour necrosis factor (PDB ID:1TNF α) with hitlist compound Acetic acid dichloro isolated from Stem + ZnONP. (e) represents the 3D image, and (f) represents the 2D image of the hydrogen bonds, respectively.

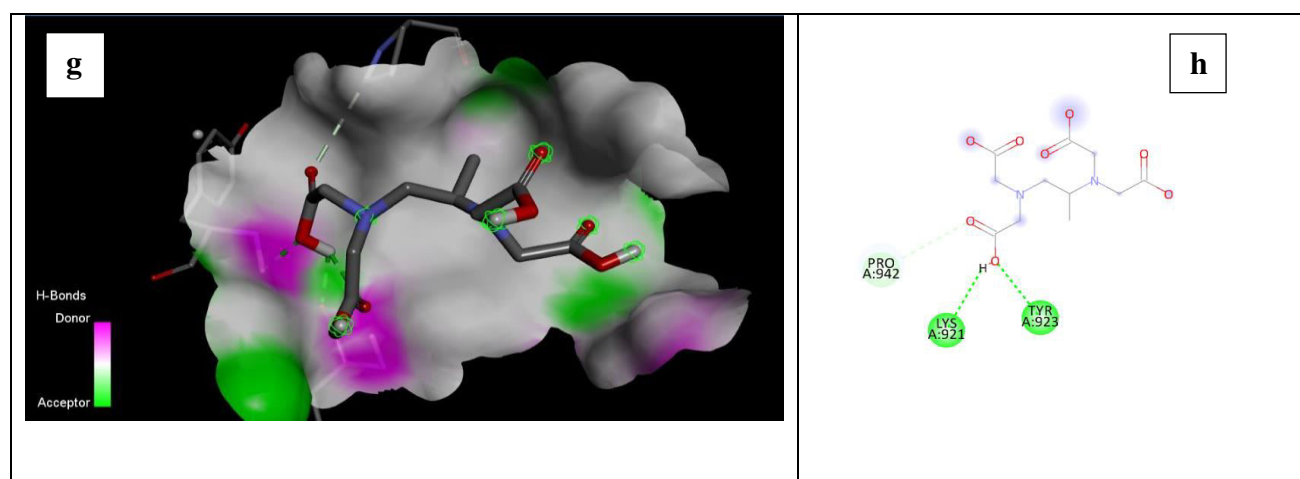


Fig.10: Illustrative representation of docking results of a best-ranked pose of major interactions with the pocket binding site of Human epidermal Growth Factor-2 (HER-2) (PDB ID:3PP0) with hitlist compound Acetic acid dichloro isolated from Stem + ZnONP. (g) represents the 3D image, and (h) represents the 2D image of the conventional hydrogen bond, respectively.

in particular when nanomaterials are fabricated for drug discovery (Hendi *et al.*, 2022). Autodock analysis makes use of a “hybrid” force field that utilized a “Full” desolvation model, along with the evaluation of hydrogen bond directivity (Sarkar *et al.*, 2022). The interaction energy is predicted in such a way that the energy of the unbound state ligand and protein is first measured, along with the energy of the protein-ligand combination. With these values as the basis, the lowest and average binding energy values are interpreted (Lahiri *et al.*, 2022).

The presence of tannins, alkaloids, flavonoids, saponins, and phenolics was documented in the seeds of *Canavalia rosea* (Aswathi and Abdussalam, 2020). The *Canavalia maritima* exhibits anti-fungal activity against the fungus *C. neoformans*, with the use of lectin isolated from the seeds of *Canavalia maritima* (Fonseca *et al.*, 2022). The bacterial (bacteriostatic) growth was found to be inhibited remarkably when treated with leaf extract of *Canavalia maritima* at minimal concentrations (Idrus *et al.*, 2021). Con M (*Canavalia maritima* lectin) is a protein that can

inhibit the growth and biofilm formation of *S. mutans* (Arruda Cavalcante *et al.*, 2013). The methanol extract of cooked *C. rosea* and fermented split beans of *C. cathartica* showed significant anticancer activity on cancer cell lines MCF-7 and HT-29 (Niveditha *et al.*, 2013).

From these earlier reports, the pharmacological aspects were verified. To understand the phytochemical constituents contributing to the medicinal values of this plant, GC-MS characterization was carried out. With the identified compounds, docking analysis was done with the specific receptor proteins Estrogen receptor- α , Tumour Necrosis factor- α , Caspase-9, and Human Epidermal Growth Factor-2.

Both the hit compounds Methane dichloro nitro and acetic acid dichloro, selected from the GC-MS analysis, upon docking studies produced the best binding interactions with the selected receptor proteins. The major compounds reported from (the MeOH extract of *C. rosea*), Methane dichloro nitro and Acetic acid Dichloro are the compounds chosen from Leaf-ZnONP and Stem-ZnONP, respectively to validate the binding interactions with protein receptors Estrogen receptor- α , Tumour Necrosis factor- α , Caspase-9 and Human Epidermal Growth Factor-2 (1A52, 1TNF α , 1NW9, and 3PP0). Both these compounds validated the best binding affinity with all the tested protein receptors, especially estrogen receptor- α , and caspase-9 protein.

Several reports of in-silico studies in the MCF-7 cell line with HER-2 receptor were highlighted. Docking studies in saponin fraction of *Tribulus terrestris* exhibited active metabolites, having anticancer properties. The study revealed the high binding affinity of Nuatigenin at significant sites with apoptotic proteins and cell surface receptors like estrogen receptor, progesterone receptor, epidermal growth factor receptor, and human epidermal growth factor receptor-2 (Patel *et al.*, 2021). Docking analysis of the bioactive compounds PH-1 (4-methyl-5-oxo-tetrahydrofuran-3-yl acetate) and PH-2 (methyl 4-

hydroxy- 3-methoxy benzoate) confirmed that cytotoxic inhibition of MCF-7, HeLa and NIH/3T3 cells was achieved by inhibition of EGFR, HER2, and VERGR receptors (Mahnashi *et al.*, 2021).

Conclusion

This investigation reports the synthesis of zinc oxide nanoparticles from the methanolic extract of the leaf and stem of *Canavalia rosea*. The methanolic fraction of *C. rosea* was subjected to GC-MS analysis, which exposed the presence of 33 and 12 total compounds in Leaf-ZnONP and Stem-ZnONP, respectively. The docking analysis was done with Methane dichloronitro and Acetic acid dichloro from leaf and stem extracts, respectively. To our knowledge, this is the foremost study involving GC-MS analysis of *Canavalia rosea*, and *in silico* procedure with several receptor proteins like Estrogen receptor- α , Tumour Necrosis factor- α , Caspase-9 and Human Epidermal Growth Factor-2 (1A52, 1TNF α , 1NW9, and 3PP0). This observation suggests that the compound could be tested further for its activity against the designated estrogen receptor (1A52), *in vitro*, to authorize the biological activity against cancer.

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